Asian Journal of Agricultural and Horticultural Research

2(1): 1-7, 2018; Article no.AJAHR.42331
ISSN: 2581-4478

Comparative Studies on Vegetative and *in vitro* Propagation of Elite Selected Jojoba Strains

A. A. Nahla[1*], M. Eliraqy[1], A. Abo El-Khasab[1], Roba M. Ismail[2], A. S. Esmail[1], S. A. A. Elsayh[1], Y. Abd El-Aziz[1], E. El-Bassel[1], H. El-Ashry[1] and M. S. Gawish[3]

[1]Horticulture Research Institute, Agriculture Research Center, Egypt.
[2]Department of Plant Genetic Transformation, Agriculture Genetic Engineering Research Institute, Agriculture Research Center, Egypt.
[3]Department of Pomology, Faculty of Agriculture, Damietta University, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. Author AAN designed the study, author HEA and performed the statistical analysis, author RMI wrote the protocol and wrote the first draft of the manuscript. Authors ASE and SAAE managed the analyses of the study. Authors ME and AAEK managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJAHR/2018/42331
Editor(s):
(1) Anita Biesiada, Professor, Department of Horticulture, Wroclaw University of Environmental and Life Sciences, ul. C.K. Norwida 25, 50-375 Wroclaw, Poland.
(2) Magdalena Valsikova, Professor, Department of Vegetables Production, Faculty of Horticulture and Landscape Engineering, Slovak University of Agriculture (SUA), Nitra, Slovakia.
Reviewers:
(1) R. Mahalakshmi, India.
(2) Sheila Jazmín Reyes Zambrano, Instituto Tecnológico de Tuxtla-Gutiérrez, Chiapas, México.
(3) Anwar Shahzad, Aligarh Muslim University, India.
(4) Fábio Henrique Portella Corrêa de Oliveira, Universidade Federal Rural de Pernambuco, Brazil.
Complete Peer review History: http://prh.sdiarticle3.com/review-history/25891

Original Research Article

Received 7th May 2018
Accepted 16th July 2018
Published 13th August 2018

ABSTRACT

Aims: Evaluate the responses of five jojoba genotypes to *in vitro* nodal segments and shoot tip culture under different growth regulators combinations.
Study Design: Comparative evaluation between vegetative and in vitro propagation of jojoba genotype.
Place and Duration: The study was carried out in tissue culture lab of fruit breeding department during 2015.
Methodology: Five selected elite genotypes of jojoba symbolled by 'C10, C16, C18, C19 and C21', and cultivated in the Cairo-Alexandria desert road were propagated asexually via cutting and

**Corresponding author: Email: nohawad@yahoo.com;*

in vitro culture technique. Two types of explants were tested; shoot tips of two months age with 0.5 to 1 cm length and semi- hard nodal segment of six months age was divided into parts with 1 to 4 cm containing from 1-2 buds after removing whole leaves except small portion to determine cutting side cultivation.

Results: Explants taking time has a great effect on jojoba plant proliferation, it was noticed that the best time for taking explants begin from the mid of March to mid of April; moreover, type of tested explants revealed different response towards proliferation. Concentrations of different growth regulators played an important role on growth and proliferation of jojoba genotypes. It was clear that combination of BA+NAA (3 mgL-1 and 0.5 mg L-1 respectively) showed highest rate of multiplication in respect to shoot number, shoot length and leaves number. Regarding rooting, proliferated plants failed to initiate roots when different types of hormones (BAP, TDZ and NAA) with different concentration (0.5, 1 and 2 mg/L) were tested. However, the tested genotypes failed to initiate any roots. On the other hand, propagation via stem cutting treated with IBA at 1500, 2500 and 3000 ppm; from these concentrations, only 3000 ppm success to initiate roots. It was clear that by increase the rooting percentage the survival percentage increase.

Conclusion: Propagation via stem cutting treated with IBA at 1500, 2500 and 3000 ppm proved that 3000 ppm concentration was the best method for obtaining the highest percentage of rooting in semi-hard wood cuttings of jojoba plants using medium comprising of peat moss, perlite and vermiculite with ratio (1:1:1); however, it is difficult to initiate roots via *in vitro* culture technique.

Keywords: Jojoba; in vitro culture; cuttings; hormones.

1. INTRODUCTION

Jojoba (*Simmondsia chinensis*) is a perennial evergreen, dioecious and an obligated cross pollinated shrub. Jojoba is cultivated in arid and semi-arid regions. The Jojoba seeds contain about 50-55% distinctive oil, which was used commercially in the cosmetic, pharmaceutical and lubricant industries. Jojoba is considered a new industrial crop compatible to the nature of Egypt desert and there is a real need to expand the cultivation area without exceeding water quota and still generate profitable economic returns.

Jojoba is propagated by sexual and vegetative methods. In plant populations derived by sexual propagation, it is difficult to determine sex type in early stages of growth and plants are genetically variable, which affects growth uniformity, physiological characteristics, yield and early bearing [1].

Several asexual methods of propagation have been used to propagate jojoba; each of these shares the major advantage over seed propagation in that they allow propagation of unique and desirable genotypes. An additional advantage of asexually propagated plants over several seedlings is that they have shorter juvenile period. Stem cuttings one of the asexual methods of propagation [2,3,4,5 and 6]. Moreover, asexual propagation via tissue culture technique suited to the multiplication of elite crops by using explants of the plant leaf, root and steam. The production of clones of known sexuality and of high oil quality and yield is necessary to ensure that commercial plantations will be productive [7]. Although vegetative propagation is an alternative way for the production of desired clones, propagation through traditional root cutting is not effective due to long procedure and slow growth [8 and 9]. Vegetative propagation methods provide genetically, uniform plant material with early fruiting. Vegetative propagation can be achieved by rooting semi–hard wood cuttings but the maximum number of possible propagules is limited by plant size and time of year [1]. On the other hand *in vitro* culture techniques reproduce true to type plants and asexual propagation of single jojoba plant is necessary to conserve the desirable traits.

The objective of this study is to propagate five elite selected jojoba strains via cuttings and *in vitro* to demonstrate the most appropriate method for vegetative multiplication.

2. MATERIALS AND METHODS

2.1 Plant Materials

Five selected elite genotypes of jojoba symbolled by ‘C10, C16, C18, C19 and C21’, and cultivated in the Cairo-Alexandria desert road were propagated asexually via cutting and *in vitro* culture technique. Two types of explants were

tested; shoot tips of two months age with 0.5 to 1 cm length and semi- hard nodal segment of six months age was divided into parts with 1 to 4 cm containing from 1-2 buds after removing whole leaves except small portion to determine cutting side cultivation. Explants were taken from the mid of February, first of March, mid of March, mid of April, first of May and mid of May and at the end of May.

2.2 Sterilization Protocol

Explants were sterilized after washing with running water for 30 min. then treated with ethyl alcohol of 90% for 10 sec. followed by a solution of Clorox concentration (15%) +2 drops of Tween 20 for 15 min. and rinsing with distilled sterilized water three times (each time 5 min.).The previous mentioned steps were followed by using mercuric chloride 0.1% for 5 min. and sterile rinsing with distilled water 3 times (5 min. each time).

2.3 Culture Medium and Plant Growth Regulators and PH Adjusting

Three different types of culture media were tested on two types of explants (semi hard nodal segment and shoot tips) as follow; (1) MS free hormones for starting, (2) MS + 2.0 mg/L BA + 0.5 mg/L NAA (3) MS+ 3.0 BA + 0.5 NAA and (4) MS + 3.0 mg/L BA + 2.0 mg/L TDZ, the last three types of media was tested for proliferation. These types of media were supplemented with 25 gm./L sucrose and 7.5 gm. / L agar and the pH was adjusted to 5.8. The media were autoclaved at 121°C and 1.05 kg cm-2 for 15 min. The maintained at 26 ± 1°C under 8h light/16 h dark photoperiod under a light intensity of 40 pmo1.m .s. A sub-culturing at 4 weeks interval was applied onto fresh medium of the same composition to preserve the cultures.

2.4 Vegetative Propagation via Stem Cutting

Semi hard cuttings were taken in the mid of March from the selected shrubs which were characterized by highest yield. These cuttings were prepared by excised of all leaves except two and each one of the cutting contains 3-5 nodes. The basal side of cut was done just below the node with 15cm. Basal portion of cutting were dipped for 10 seconds in IBA solution at 1500, 2500 and 3000 ppm and planted in mixture of Peat moss perlite and vermiculite 1:1:1 under mist propagation to study the percentage of rooting / cuttings, number of roots/cutting [4,5 and 6].

2.5 Statistical Analysis

Each treatment was performed in six jars containing five explants and each experiment was replicated three times. Data were subjected to analysis of variance at the end of the experiment (eight weeks) by MSTAT-C [10] Computer statistical analysis program. LSD, test at the 5% level of significance (P=0.05).

3. RESULTS AND DISCUSSION

Explants taking time has a great effect on jojoba plant proliferation, it was noticed that the best time for taking explants begin from the mid of March to mid of April; otherwise, explants taken at mid-February, first of March and mid of May failed to proliferate.

Type of tested explants revealed different response towards proliferation (Fig. 1), Semi-hard woody stem was the most preferable explants; meanwhile, shoot tips failed to survival. Plant genotype *and in vitro* applied auxins had prime role on the survival of plantlets during acclimatization [11]. A nodal segment was used for in vitro propagation of jojoba plant; the sprouting percent is high when used nodal segment as explant [12]. DKW basal medium supplemented with various concentrations of BAP were used, BAP alone induced 15 shoots per original seedling explants over a 12 week period at a concentration of 4.0 mg L-l [13]. The juvenility of the explants probably contributed to this high proliferation rate.

3.1 Effect of Jojoba Plant Genotype and Plant Growth Regulators on Proliferation

Concentrations of different growth regulators played an important role on growth and proliferation of jojoba genotypes. It was clear that combination of BA (3mg/L) and NAA (0.5 mg/L) showed highest rate of multiplication in respect to shoot number, shoot length and leaves number as shown in Table 1. Meanwhile, BA+ NAA at 2 mg L^{-1} and 0.5 mg L^{-1} respectively had least efficiency on shoot number. Combination of 3mg L^{-1} BA+ 2mg L^{-1} TDZ yield higher leaves number and shoot length in compare to combination of 2mg L^{-1} BA+ 0.5 mg L^{-1} NAA. Regarding

genotype effect on the proliferation, it was clear that genotype C18 showed highest records of vegetative parameters (shoot number and shoot length) irrespective of the tested hormones. On the other hand, C10 and C16 revealed the highest proliferation of shoot numbers.

'Al-Maddenah' cultivar conveyed the highest response to nodal segments, with MS culture medium supplemented with BAP (3 mg/L) alone and BAP (3 mg/L), in combination with IAA (7 mg/L). Modifying sucrose concentration in the culture medium (20 g, 40 g, 50 g and 60 g) enhanced shoot formation of nodal segments of the genotype 'Hada Al-Sham". Concerning shoot tip culture, explants of the genotype 'Hada Al-Sham' were cultured on MS+BAP (5 mg)+IAA (5 mg) produced the highest percentages of bud sprouting and shoot formation [14]. However, [15] indicated that BAP at 0.5 mg/L gave the best sprouting percent. BAP was better than TDZ in improving shoot and leaf numbers as well as shoot length while, TDZ stimulated callus production more than BAP. Highest number of shoots (9.13 shoots per explant) was obtained on MS medium containing 2.0 mg/L BAP [16].

Regarding rooting, proliferated plants failed to initiate roots when different types of hormones (BAP, TDZ and NAA) with different concentration (0.5, 1 and 2 mg/L) were tested. However, the tested genotypes failed to initiate any roots.

Table 1. Effect of jojoba plant genotype and plant growth regulators on proliferation after six weeks of proliferation

Growth regulators / Genotype	2.0 BA + 0.5 NAA			3.0 BA + 0.5 NAA			3.0 BA + 2.0 TDZ		
	Shoot number	Leaves number	Shoot length	Shoot number	Leaves number	Shoot length	Shoot number	Leaves number	Shoot length
C10	1.0	4.0	10.0	3.9	4.5	10.90	2.3	4.0	11.90
C16	1.5	2.8	12.80	2.6	4.9	11.90	1.0	4.0	12.20
C18	2.3	3.0	12.60	4.1	3.9	12.80	2.8	2.6	13.80
C19	1.2	3.0	9.20	3.3	2.6	10.10	2.0	2.6	11.80
C21	1.9	3.2	11.10	3.9	3.3	12.70	1.8	4.0	10.70
L.S.D at 0.5	0.12	0.23	1.1	1.4	0.99	2.2	0.12	1.9	2.1

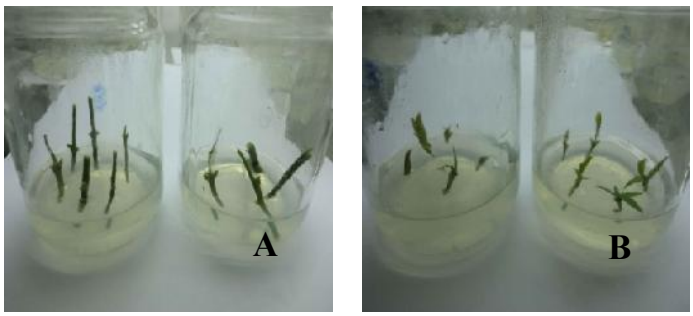

Fig. 1. Nodal segments (A) and shoot tip (B) of jojoba used as explants cultured on MS media free hormones

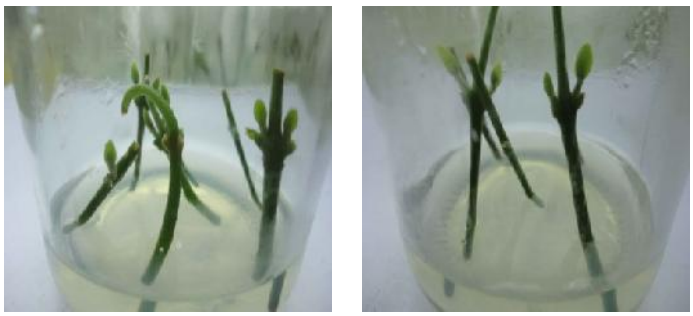

Fig. 2. Proliferation of jojoba nodal segments after two weeks cultured on MS media supplemented with 2.0 BA + 0.5 NAA

Fig. 3. Proliferation of jojoba nodal segments after six weeks cultured on MS media supplemented with 3.0 BA + 2.0 TDZ

Table 2. Growth parameters jojoba cuttings as affected by strain genotype

Strain no.	Rooting %	No. of roots / cutting	Length of primary root (cm)	Diameter of primary root (mm)	Length of primary shoots (cm)	Diameter of primary shoot (mm)	Survival %
C10	32.10	12.35	5.12	0.34	4.12	1.19	31.1
C16	38.15	15.36	6.13	0.53	5.18	1.21	37.2
C18	42.34	19.78	8.21	0.71	6.24	1.05	45.1
C19	36.15	15.12	7.81	0.60	4.23	1.17	36.2
C21	39.12	18.19	5.23	0.45	5.92	1.32	39.4
L.S.D 5%	2.19	1.01	1.19	0.10	2.15	0.32	3.05

Fig. 4. Vegetative propagation of jojoba via semi hard stem cuttings in medium comprising peat moss, perlite and vermiculite

3.2 Characterization of Jojoba Cutting Parameters Resulted from Vegetative Propagation

Rooting parameters were affected by the genotype of the tested strains and the tested concentrations of IBA, e.g., low and medium concentrations of IBA (1500 and 2500 ppm) fail to initiate any roots. Meanwhile, the high concentration (3000 ppm) of IBA the only one which enhancing roots initiation.

Cuttings from strain C18 recorded the highest value of rooting percentage, number of roots, and length of primary root and diameter of primary root. Meanwhile, strain C10 showed a lowest record regarding the same parameters of root. On the other hand, C16, C19 and C21 exhibited an intermediate value of root parameters (Table 2). In this respect, [17] related the rooting rates of jojoba cuttings to age of mother plant, temperature and humidity as the rooting rates of cuttings from young and old female jojoba shoots were 60 - 64 and 3-5%, respectively at a temperature of 19-23°C and 80-85% R.H. [18] indicated that the rooting ratio of young jojoba individual was higher than mature individuals. Clones with a high rooting

percentage also tended to produce an increased number of roots per cutting [19]. Regarding primary shoot length data obtained from strains C10 and C18 detected the lowest and highest value, respectively. However, lowest score of primary shoot diameter was observed by strain C18 and the highest score was observed by strain C21.

It was clear that by increase the rooting percentage the survival percentage increase, similar findings were reported by [20] in semi-hardwood cuttings of guava.

Variability among jojoba plants may also play a role in rooting potential, although it is not as important as season. Selecting plants with naturally high rooting potential could make it easier to root cuttings and might eliminate the need to collect cutting material at specific times of the year. In general, the use of auxin on jojoba cuttings during periods of high rooting potential promotes adventitious root formation, but during periods of low rooting potential it has no effect or is even slightly inhibitory.

4. CONCLUSION

Propagation via stem cutting treated with IBA at 1500, 2500 and 3000 ppm proved that 3000 ppm concentration was the best method for obtaining the highest percentage of rooting in semi-hard wood cuttings of jojoba plants using medium comprising of peat moss, perlite and vermiculite with ratio (1:1:1); however, it is difficult to initiate roots via *in vitro* culture technique.

ACKNOWLEDGEMENT

This research project was carried out in Agricultural Genetic Engineering Research Institute and funded by the Science and Technological Development Fund (STDF) under the Contract No. 4972 titled “Jojoba (Simmondsiachinensis) Plant as a Novel Source for Energy Production”.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Eed AM, Burgoyne AH. Biotechnology of jojoba (*Simmondsia chinensis*): Progress and difficulties. 3rd International Conference on Biotechnology Applications in Agriculture (ICBAA), Benha University, Moshtohor and Sharm El-Sheikh- Egypt; 2016.
2. Reddy SJ, Cardran PA, Hogan L, Palzkill DA. Recent advances in vegetative propagation of jojoba by cuttings. Proceedings of the 4 International Conference on Jojoba and its Uses, Hermosillo Sonora (Mexico). 1980;103–8.
3. Palzkill DA. Propagation of jojoba by stem cuttings. Proceedings of the 7th International Conference on Jojoba and its Uses, Phoenix, Arizona (U.S.A.). 1988;86–101.
4. Ayerza R, Prineen LH. Rossi evaluation of eight jojoba clones for rooting capacity, plant volume, seed yield, and wax quality and quantity. Proceedings of Ninth intern. Conf. on Jojoba and its uses and of the 3rd intern. – Conf. on new industrial crops and products. 1996;1–3.
5. Basher MA, Iqbal AM, Asad MN, Rana MY, Shah SFH. Propagation of jojoba through cuttings. J. Anim. Pl. Sci. 2001;11:24-9.
6. Hamad A. Physiological studies on Jojoba Plant PhD. Thesis. Pomology Faculty Of Agriculture Benha University; 2010.
7. Kumar S, Mangal M, Dhawan AK, Singh N. Biotechnological advances in jojoba [Simmondsia chinensis (Link) Schneider]: Recent developments and prospects for further research. Plant Biotechnol Rep. 2012;6:97–106.
8. Turgut-Kara N, Arı Ş. Micropropagation of astragalus maximus willd, biotechnology & biotechnological equipment. 2006;20(1): 20-22.
9. Singh A, Reddy MP, Patolıa JS. An improved protocol for micro-propagation of elite genotypes of *Simmondsia chinensis* (Link) Schneider. Biologia Plantarum. 2008;52(3):538-542.
10. Mstat C. Microcomputer statistical program for experiment design and analysis. MSTAT / Michigan State University, Michigan, USA; 1990.
11. Bashir MA, Rashid H, Anjum MA. *In vitro* shoot initiation from nodal explants of jojoba (*Simmondsia chinensis*) strains. Biotechnololgy. 2007;6:165-174.
12. Taha RA, Taha LS, Metwally SA. *in vitro* cultures of jojoba (*Simmondsia chinensis* L.) affecting by laser irradiation. J Chemi Biol Phys Sci, Sec B. 2015;5(4):3906-3913.
13. Roussos PA, Tolia MA, Pontikis CA, Kotsias D. Rapid multiplication of jojoba

seedlings by *in vitro* culture. Plant Cell, Tissue and Organ Culture. 1999;57(2):133-137.
14. Fayek MA, Shaaban EA, El-Obeidy AA, Taha RA. *In vitro* propagation of three female jojoba clones (*Simmondsia chinensis* Link Schneider). Egypt J App Sci. 2007;22(6B):652-665.
15. Rania A, Hassan SAM, Dorria MM, Nagwa S. A comparative study on different cytokinin types and carbon source concentrations on *in vitro* proliferation of jojoba (*Simmondsia chinesis* Link (Schneider). International Journal of ChemTech Research. 2016;9(08):178-184.
16. Öncel Z, Erişen S. Clonal propagation of jojoba by *in vitro* culture and determination of sexuality of the regenerates. The J. Anim. Plant Science. 2017;27(2):567-574.
17. Zhou Y. A preliminary study on propagation by cutting of *Simmondsia chinensis* (in Chinese). Plant Physiol. Communic. 2002;38:564-566.
18. Cao B, Gao HD. Technology of cutting propagation of *Simmondsia chinensis* (Link) Schneider (in Chinese). J. Nanjing Forest. Univ. 2003;27:62-66.
19. Lee CW, Palzkill DA. Propagation of jojoba by single node cuttings. Hort. Science. 1984;19:841-42.
20. Luqman M, Ali N, Sajid M. Effect of different concentrations of indolebutyric acid (IBA) on semi-hardwood guava cuttings. Sarhad J. Agric. 2004;20:219-222.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/25891